MORPHOLOGIC STUDIES

Intravascular Ultrasound Imaging: In Vitro Validation and Pathologic Correlation

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Intravascular ultrasound imaging is a new method in which high resolution images of the arterial wall are obtained with use of a catheter placed within an artery. An in vitro Plexiglas well model was used to validate measurements of the luminal area, and an excellent correlation was obtained. One hundred thirty segments of fresh peripheral arteries underwent ultrasound imaging and the findings were compared with the corresponding histopathologic sections. Luminal areas determined with ultrasound imaging correlated well with those calculated from microscopic slides (r = 0.98).

Three patterns were identified on the ultrasound images: 1) distinct interface between media and adventitia, 2) indistinct interface between media and adventitia but different echo density layers, and 3) diffuse homogeneous appearance. The types of patterns depended on the relative composition of the media and adventitia. Calcification of intimal plaque obscured underlying structures. Atherosclerotic plaque was readily visualized but could not always be differentiated from the underlying media.

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Intravascular ultrasound imaging is a new technique for visualizing arterial structure (1–3). A high frequency ultrasound transducer on the tip of a catheter inserted directly into a blood vessel can obtain reproducible high resolution images. Use of intravascular ultrasound in atherosclerotic arteries has the potential to delineate the absolute luminal area, the structure of the arterial wall and the presence and extent of atherosclerotic plaque.

Although there have been recent reports (4–9) about the feasibility of using an intravascular ultrasound catheter both in vitro and in vivo, limited information has been provided concerning the ultrasound appearance of arterial structures compared with findings on histologic examination (10–13). The purpose of this study was to examine the ultrasound images taken from a wide variety of different pathologic arterial segments to provide a basis for interpreting the ultrasound images.

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Methods

Ultrasound transducer. The intravascular ultrasound catheter used in this study comprises a 20 MHz transducer mounted on the tip of an 8F catheter (CVIS). The ultrasound beam is reflected onto a rotating mirror revolving at 1,500 rpm, creating a 360° real-time image perpendicular to the catheter. The resolution of the image is <1 mm, and the radius of penetration is ≤4 cm. The beam width is 1 mm at a 2 mm depth. A long cable throughout the length of the catheter is used to drive the mirror and is connected to a mechanical motor at the very distal end of the catheter. A plastic housing surrounding the transducer and mirror is filled with physiologic solution to allow penetration of the ultrasound beams. The real-time images were all recorded on 0.5 in. (1.27 cm) videotape for future analysis.

In vitro model correlation. A phantom Plexiglas model was created in which fluid-filled wells of a known dimension were drilled (Fig. 1). The wells were 10, 14, 16, 20, 26 and 30 mm in diameter. The tip of the transducer was immersed in the wells to obtain an image of well perimeter. Measurements were obtained with the catheter positioned in the middle of each well and eccentric toward the side of the well. Measurements were also obtained with the catheter in the coaxial and "off-axis" position.

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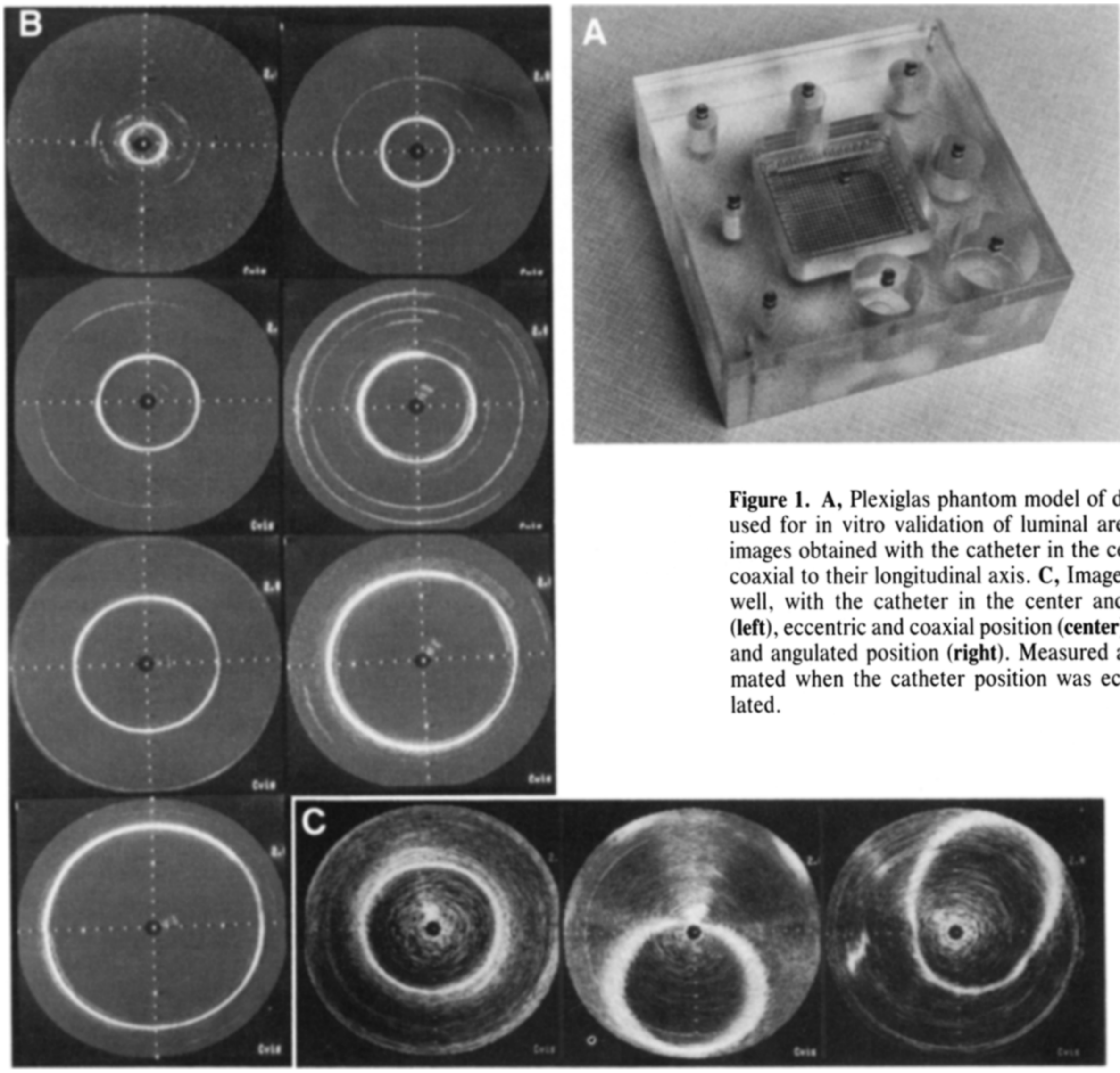

Figure 1. **A,** Plexiglas phantom model of different well sizes used for in vitro validation of luminal area. **B,** Ultrasound images obtained with the catheter in the center of the wells, coaxial to their longitudinal axis. **C,** Images from the 20 mm well, with the catheter in the center and coaxial position (**left**), eccentric and coaxial position (**center**) and the eccentric and angulated position (**right**). Measured area was overestimated when the catheter position was eccentric and angulated.

Pathologic correlation. The ultrasound images taken from fresh postmortem arterial specimens were compared with findings on corresponding microscopic sections. There were 12 patients aged 20 to 82 years. Fifteen arteries were used to provide a wide range of arterial types: distal abdominal aorta and common iliac, external iliac, femoral, renal, subclavian, mesenteric and carotid arteries. A total of 130 individual segments were selected from these arteries for ultrasound and microscopic evaluation.

Arterial specimens were collected at the time of autopsy, and all were imaged fresh, without fixation and within 4 h of collection. Surrounding soft tissue was dissected from each artery. Small arterial perforators and branches were tied off with sutures, and the distal end of the artery was occluded with a large cork. A 8F sheath was sewn into the proximal end of the artery to complete the closed system. Physiologic saline solution was infused through the side arm of the sheath. The pressure inside the artery was maintained at a physiologic level (60 to 80 mm Hg) with a syringomanometer connected to the infusate.

The intravascular ultrasound catheter was then inserted through the diaphragm of the sheath. The catheter was aligned coaxial to the arterial segment, and the entire arrangement was immersed in a saline bath, which was kept at room temperature.

Images were then taken at 1 cm intervals along the entire length of the artery. The gain and contrast were adjusted for each image to provide optimal image quality. The position of the ultrasound transducer was indicated by measuring the length of the inserted portion of the catheter and marking this position in ink on the specimen to ensure that histopathologic sections were obtained from the same location as the ultrasound images.

Arteries were then pressure perfusion-fixed in 10% neu-

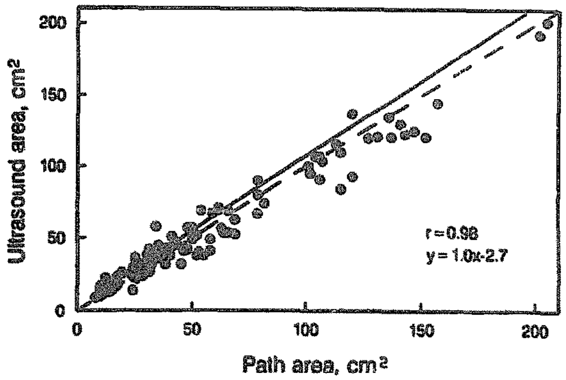

Figure 2. Correlation of luminal areas of histologic specimens (Path) obtained from histologic sections with areas obtained from ultrasound images.

tral buffered formalin. After fixation for 24 h, the arteries were removed and placed in a standard decalcification solution. Sections taken at the 1 cm markers were then processed routinely for light microscopy and stained with hematoxylin-eosin and elastic-Van Gieson's stains.

The intravascular ultrasound images from the 0.5 in. (1.27 cm) videotape were digitized onto a 256 × 256 matrix on a computer (Dextra) dedicated for echocardiographic analysis. Therefore, the analysis was performed on stop frame images taken from the real-time images. The absolute luminal area was determined by planimetry with previously validated software. After the area measurement, the thicknesses of the different "layers" that appeared on the ultrasound image were measured. The relative thickness of each layer was used for comparing the ultrasound structures with the histopathologic specimens.

The histopathologic specimens were photographed and enlarged to 8 × 10 in. (20.3 × 25.4 cm) prints. The absolute luminal area on the prints was then determined by planimetry with the same digitization board used for the ultrasound images. The planimetric luminal areas on the ultrasound images were then compared with those of the histopathologic specimens. A simple linear regression analysis was used for this comparison.

The microscopic specimens were then analyzed under light microscopy by a cardiovascular pathologist (W.D.E.). Arteries were analyzed specifically for type of artery (that is, elastic, musculoelastic or muscular), composition of the media and adventitia, appearance of the internal and external elastic lamina and composition of existing atherosclerotic plaque. The relative thicknesses of the layers on the ultrasound images were compared with those of the histopathologic arterial layers to determine whether the ultrasound layers accurately corresponded to morphologic arterial subunits. These comparisons were made by two independent observers (R.A.N. and C.A.W.).

Table 1. Ultrasound Appearance of Media and Adventitia

Pattern	Description
1	Distinct interface between media and adventitia
2	Indistinct interface between media and adventitia; different echo density of media and adventitia
3	Homogeneous appearance of media and adventitia

Results

In vitro validation. The ultrasound catheter system was validated by immersing the transducer tip in fluid-filled wells of known luminal dimension (Fig. 1A and 1B) and recording the images obtained on 0.5 in. (1.27 cm) videotape. Digitized stop frame images were measured with the same computer program used for analysis of the dimension and area of the pathologic arterial specimens. For well sizes of 10, 14, 16 and 20 mm in diameter, the ultrasound catheter-derived measurements were identical to the well sizes, expressed to the nearest millimeter. For well sizes of 26 and 30 mm in diameter, catheter-derived measurements of 27 mm and 31 mm, respectively, were obtained.

Dimensional measurements of the wells were identical whether the catheter was central or eccentric within the well as long as the axis of the catheter was parallel to the longitudinal axis of the well. When the catheter was eccentric, there was a "blooming" effect, in which the well wall had a thick echo-dense appearance on the ultrasound image. When the catheter was positioned off axis, the circular shape was distorted to an elliptic one, as would be expected because the plane of ultrasound was no longer perpendicular to the sides of the well (Fig. 1C). In the case of a 20 mm well size and an estimated off-axis angle of 30°, the well dimension increased by 30% (long axis of the elliptic image), and the area was overestimated by 20%.

Luminal area. The correlation between the planimetric luminal area on the ultrasound images and the area obtained from histologic images is shown in Figure 2. The correlation coefficient was 0.98.

Table 2. Patterns of Media and Adventitia in the Various Types of Arteries

	Type of Artery (no.)		
Pattern	Elastic	Transitional	Muscular
1	Aorta (21)	Common iliac (16)	Common iliac (4)
	Common iliac (5)	External iliac (3)	External iliac (11)
2	Subclavian (2)	Common iliac (3)	—
	Carotid (4)	External iliac (4)	
3	Carotid (3)	Common iliac (20)	External iliac (15)
		External iliac (3)	Mesenteric (3)
		Renal (4)	Femoral (2)

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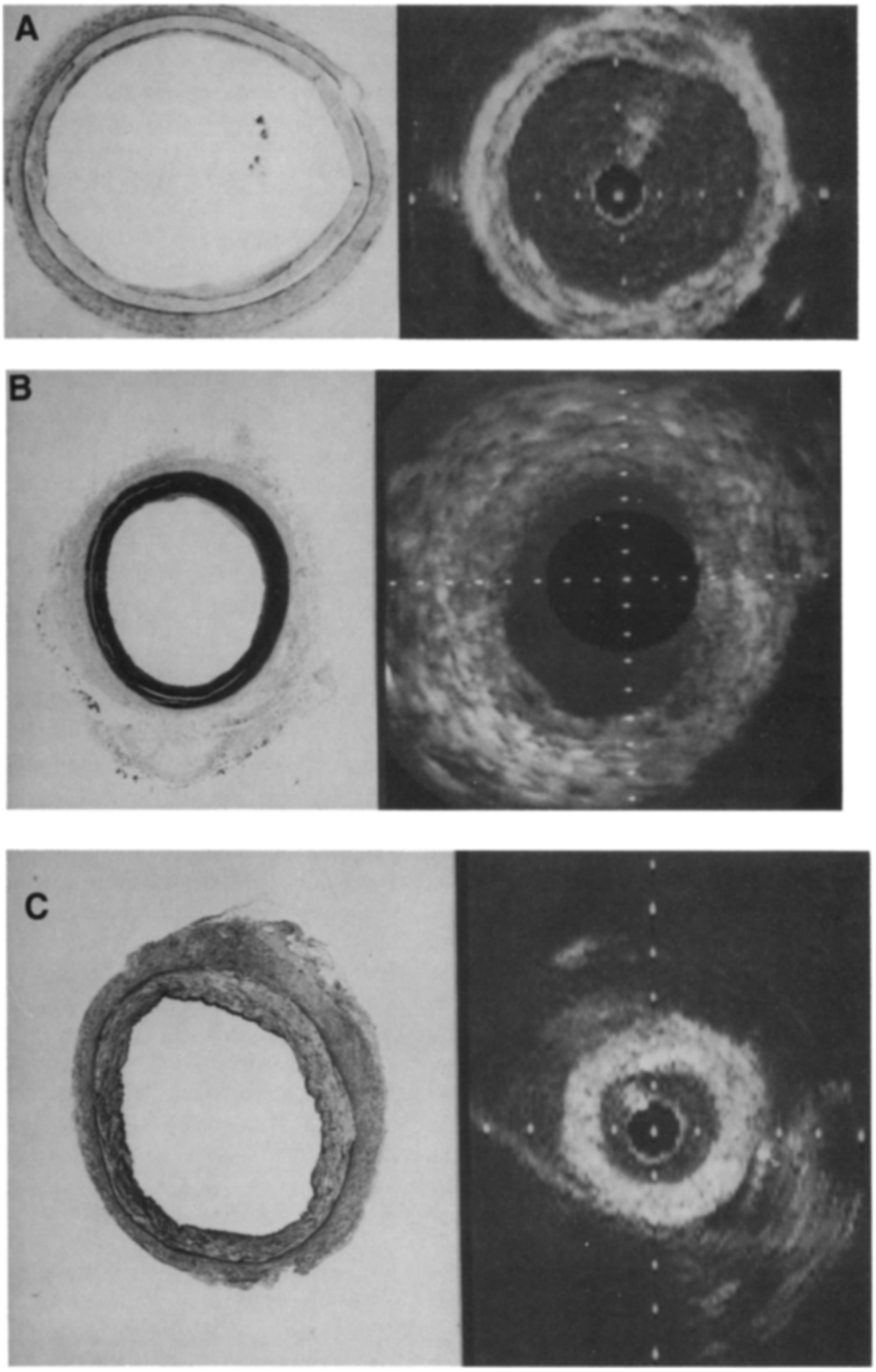

Figure 3. The three patterns of media and adventitia. **Left panels,** Histologic specimen; **right panels,** Ultrasound image. **A,** Common iliac artery with distinct interface between media and adventitia (pattern 1). **B,** Carotid artery with indistinct interface but differing ultrasound densities of media and adventitia (pattern 2). **C,** External iliac artery with homogeneous appearance (pattern 3).

Definition of Media and Adventitia (Tables 1 and 2)

Of the 130 arterial segments analyzed microscopically, 40 were from elastic arteries, 55 from transitional (musculoelastic) arteries and 35 from muscular arteries. Three different patterns emerged on the ultrasound images when the media and adventitia were examined (Table 1, Fig. 3).

Elastic arteries. Of the 40 elastic arteries examined microscopically, 5 segments had dense calcification of intimal

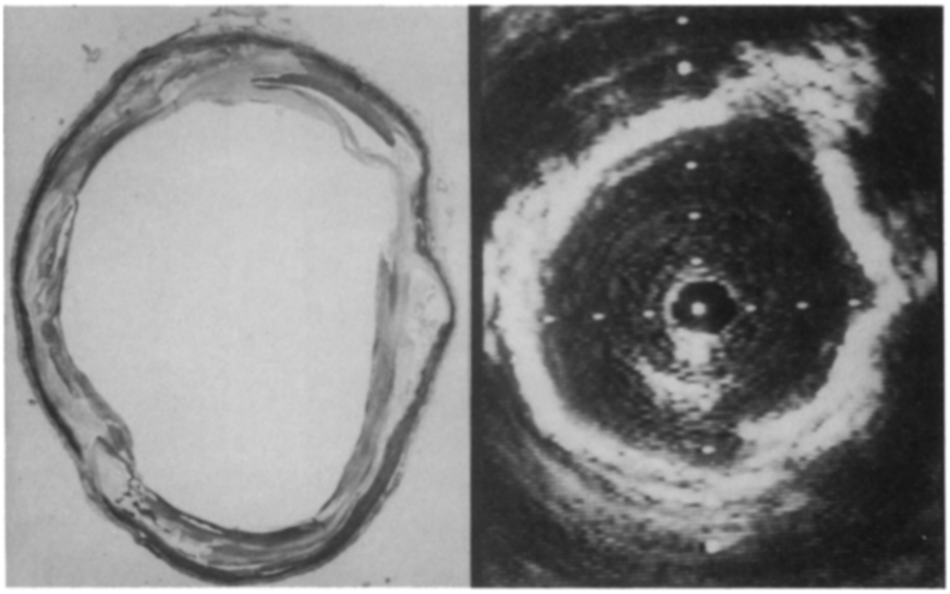

Figure 4. Distal abdominal aorta with diffuse, dense calcification in intimal atheroma. **Left,** Histologic specimen; **right,** Ultrasound image. There is "shadowing" of the ultrasound beam so that the underlying structures are not observed.

atheroma. The corresponding area on the ultrasound image was so highly echo reflective that there was diffuse "shadowing" of the ultrasound beam in which no image was present outside of the echo-dense inner layer. Therefore, the media and adventitial layers could not be visualized (Fig. 4).

In 26 of the remaining 35 segments, a distinct interface was visualized between the media and the adventitia. Microscopically, the media consisted primarily of elastin and the adventitia was loosely collagenous with little or no smooth muscle (Fig. 5, top). Thus, there was a compositional difference and a distinct line of demarcation between the two layers. Corresponding ultrasound images demonstrated a dark homogeneous inner layer, representing the media, and a lighter homogeneous outer layer, representing the adventitia (pattern 1). This pattern was present in elastic arteries containing loose adventitial collagen, such as in the aorta and common iliac arteries.

In 9 of the 35 segments, the media consisted of denser elastin, and denser collagen was present in the adventitia, as seen in the carotid and subclavian arteries (Fig. 5, bottom). Consequently, there was no distinct interface between the media and adventitia in these segments on the ultrasound images. In six of the nine segments, there was an inner media that had a lighter appearance than the darker outer adventitial layer (pattern 2). Three of the nine segments had a completely homogeneous pattern without any layers visible (pattern 3).

Transitional arteries. The ultrasound patterns in 55 segments of transitional arteries (including common iliac, proximal external iliac and renal) were evaluated. The media in these arteries consisted of both elastin and smooth muscle. In two segments, significant circumferential calcification caused a marked reflectance of the ultrasound beams, with shadowing, as described previously. In these two segments, the media and adventitia were not visualized.

In 19 of the remaining 53 segments, the adventitia contained elements similar to the media and consisted of smooth muscle, elastin and collagen. However, because the smooth muscle was located in the outer layer of the adventitia, a distinct interface between media and adventitia was easily recognized microscopically (Fig. 6, bottom). Moreover, the ultrasound image also demonstrated a distinct interface between media and adventitia (pattern 1). In 11 of these 19 segments, the media had a light appearance, in contrast with the dark adventitial layer. In the other eight segments, there was a dark line interface between the two layers.

In 34 of the remaining 53 segments, the adventitia consisted of approximately half smooth muscle and half collagen. In these segments, the smooth muscle was diffusely located throughout the entire adventitia. The elastin located in the media also appeared to extend into the adventitia (Fig. 6, top). Thus, microscopically, there did not appear to be a distinct interface between media and adventitia, even if an external elastic lamina was identified. Similarly, all of the ultrasound images had a homogeneous appearance. In 7 of the 34 segments, the inner layer was slightly lighter than the outer layer, but no distinct interface was present (pattern 2). The remaining 27 segments had a completely homogeneous pattern without any layers (pattern 3).

Muscular arteries. Thirty-five segments of muscular arteries (distal common iliac, external iliac, femoral and mesenteric) were analyzed. In 15 of these segments, smooth muscle was either absent from the adventitia or occupied only its outer fringes (Fig. 6, bottom). Therefore, the inter-

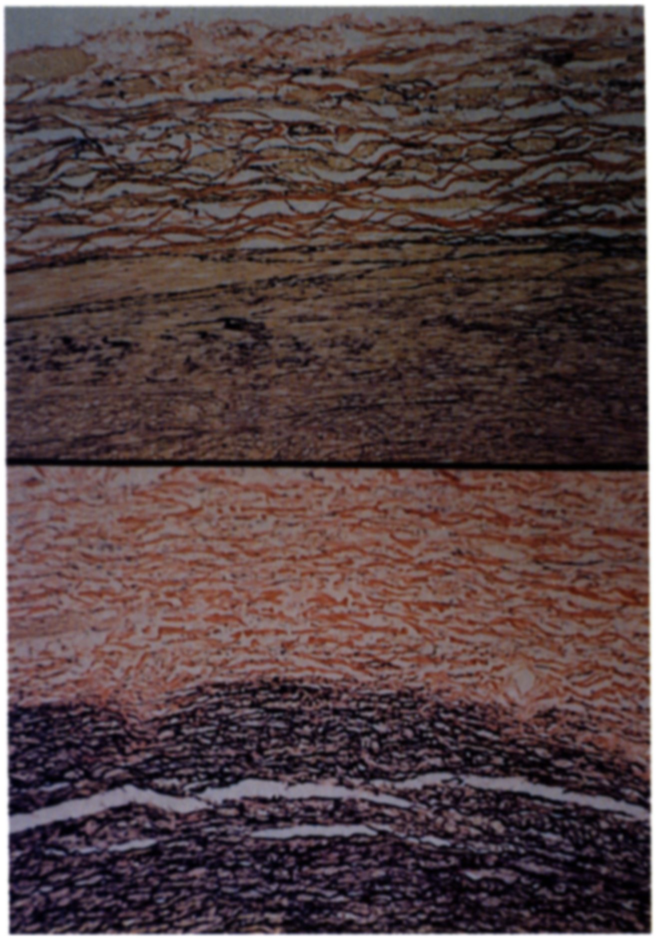

Figure 5. Histologic specimens of elastic arteries. **Top,** Common iliac artery with loose collagen and little smooth muscle in adventitia. **Bottom.** Carotid artery with dense elastin and dense collagen in media (elastic-Van Gieson's stain; original magnification ×10, reduced by 26%).

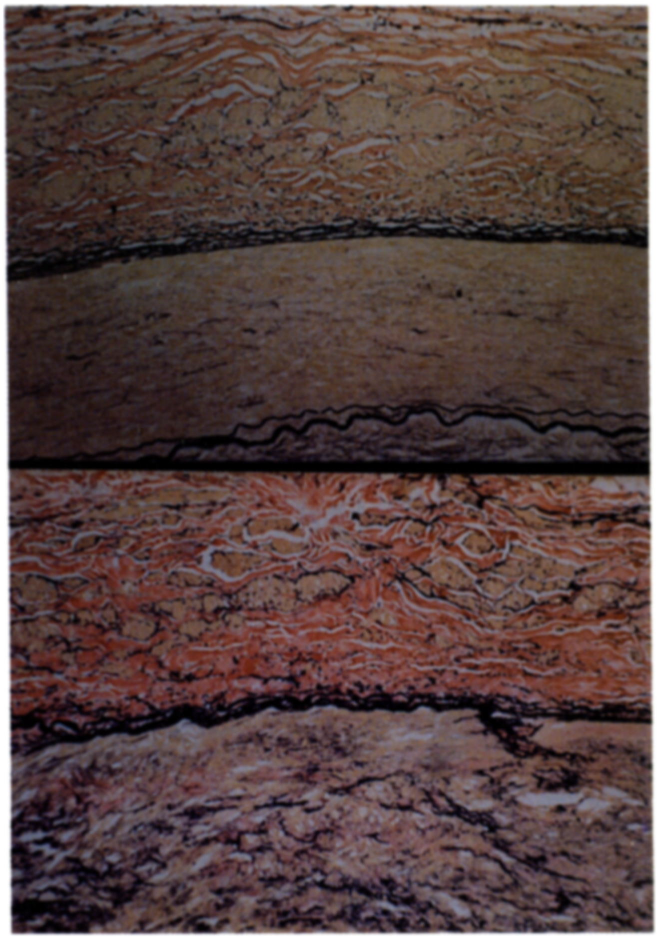

Figure 6. Histologic specimens of transitional or muscular arteries. **Top,** External iliac artery with dense amounts of smooth muscle throughout entire adventitia. **Bottom,** Common iliac artery with smooth muscle located in the outer portion of the adventitia (elastic-Van Gieson's stain; original magnification ×10, reduced by 26%).

face between media and adventitia was distinct on both microscopic and ultrasound examination (pattern 1).

The remaining 20 segments had smooth muscle diffusely scattered throughout the adventitia, intermixed with collagen (Fig. 6, top). As a result, adventitial smooth muscle blended with that in the media. This was seen in the external iliac, femoral and mesenteric arteries. The ultrasound appearance was diffusely homogeneous (pattern 3). There was no pattern 2 seen in the muscular arteries.

Composition of other ultrasound layers. To define the composition of several ultrasound appearances, repeat imaging was performed after manipulation of the pathologic specimens. The adventitial layer had been dissected from several of the elastic arteries (aorta) so that the specimen consisted almost entirely of all elastic media. The ultrasound images in these arteries consisted of three layers: a thin inner echo-dense layer, a thick black middle layer and a thin outer echo-dense layer (Fig. 7). These thin echo-dense layers at the boundaries were considered to represent the interface between the tissue and surrounding saline solution.

In six muscular arteries, the entire artery was of a homogeneous pattern, but a thin black line was located in the inner one third of the arterial wall (Fig. 8, left). To further elucidate the location of this thin black line, the media was artificially dissected from the adventitia (Fig. 8, right). This demonstrated that the black line was the media as it interfaced with the adventitia, highlighted by bright echoes from the inner layer of the media.

Intimal Visualization

Among the 130 arterial segments studied microscopically, 89 exhibited intimal atherosclerotic disease. The 41 remaining segments had a normal intima that was very thin and could not be visualized on the ultrasound images.

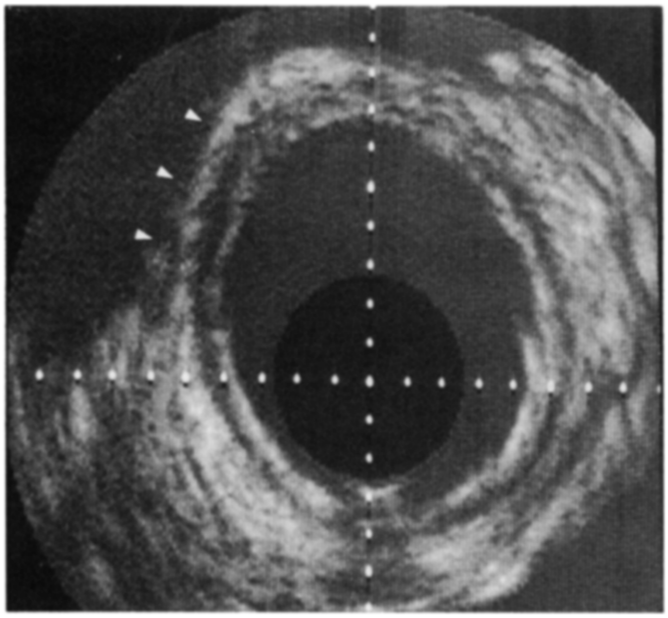

Figure 7. Ultrasound image of aorta in which adventitia has been completely dissected (**arrowheads**), demonstrating a thin outer echo-dense layer, a thick black middle layer and a thin inner echo-dense layer.

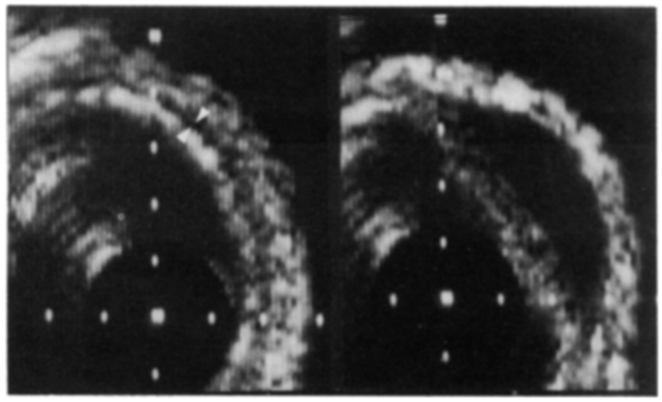

Figure 8. Left, Muscular external iliac artery demonstrating a thin black line in the inner one third of the arterial wall (**arrowheads**). **Right,** Dissection of media and adventitia showing that the thin black line is the outer media as it interfaces with the adventitia.

In 25 of the 89 atherosclerotic arteries, calcification was present in the intimal plaque. This resulted in a high degree of echo reflectance; thus, the shadowing that was present obscured the underlying structures. In the distal abdominal aorta, calcification and shadowing were seen along the entire perimeter of the intima (Fig. 4), whereas in the smaller arteries the shadowing was visualized in only discrete areas, corresponding to small calcific plaques (Fig. 9).

Five specimens had a distinct interface between the atherosclerotic plaque and the underlying media (Fig. 10). Three of these segments contained a fibrous plaque with a well defined internal elastic lamina. Two segments contained a soft necrotic plaque composed mainly of cholesterol crystals. Distinct interfaces were observed in transitional and muscular arteries. In 57 segments, however, there was no distinct interface between the atherosclerotic plaque and the underlying media (Fig. 11). Among these were 52 dense fibrous plaques and 5 softer plaques with both fibrosis and cellular proliferation. This pattern was seen in elastic, transitional and muscular arteries.

Two segments were involved by rupture of a soft necrotic plaque and secondary luminal thrombus formation. These large thrombi, 6 mm in diameter, were visualized on the ultrasound image as an echo density with less echo reflectance than any other structure described (Fig. 12).

Discussion

The ability to assess vessel wall morphology and luminal characteristics of diseased arteries may potentially have valuable applications in the field of cardiovascular medicine. It is important to be able to determine the severity of atherosclerotic obstruction, for which the current method of angiography has many limitations (14,15). The nature of an atherosclerotic obstruction may provide important prognos-

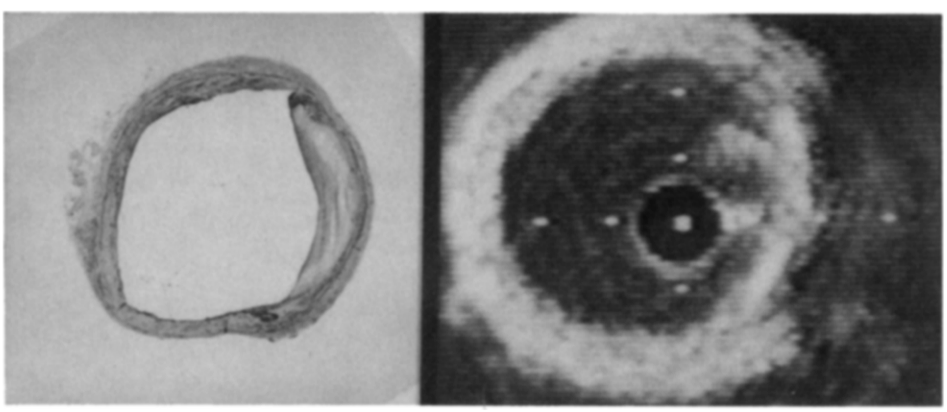

Figure 9. Calcification of intimal plaque in iliac artery resulting in shadowing in one discrete area on the ultrasound image. **Left,** Histologic specimen; **right,** Ultrasound image.

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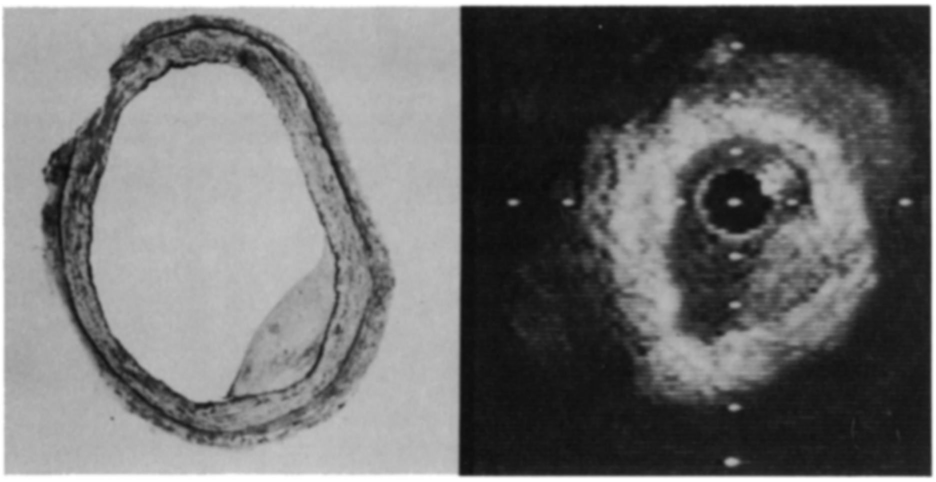

Figure 10. Discrete interface between atherosclerotic plaque and underlying media in iliac artery. **Left,** Histologic specimen; **right,** Ultrasound image.

tic implications about whether an interventional procedure, such as angioplasty or atherectomy, might be successful. In addition, certain characteristics of an atherosclerotic lesion, such as the presence of an ulcerated plaque or a thrombus, may have important implications for the immediate prognosis of a patient (16).

Angioscopy has been recently developed in an attempt to visualize the composition of atherosclerotic obstructions (16,17). One limitation of angioscopy is the fact that a blood-free environment must be created to image the artery, necessitating complete occlusion proximal to the lesion. In addition, angioscopy is able to visualize only the surface of the vessel and does not provide information concerning quantitation of the size of the vessel or the composition of the arterial wall.

Intravascular ultrasound imaging may provide the method by which these limitations can be overcome (1–3). Because circulating blood is an excellent media for transmission of ultrasound waves, real-time images can be obtained under physiologic conditions. Moreover, because a relatively short length of visualization is required when imaging from inside a vessel, high frequency transducers can be used to obtain high resolution images. These images not only have the advantage of examining the surface of the vessel but also provide information about the morphology of the entire vessel wall and plaque. Although preliminary studies have shown that it is feasible to obtain intravascular ultrasound images both in vitro and in vivo, data are limited about what the various ultrasound images represent (10,13).

Luminal area. As in other studies (3,6,7,9,11,12,18), this investigation showed that the ultrasound imaging catheter can provide accurate measurements of luminal area in vivo and in pathologic specimens. There was a small deviation from the line of identity in the pathologic specimens, which was most likely a result of a difference in the size of the vessel between the ultrasound measurement and the pathologic measurement. Perfusion of arteries in the freshly excised state may have resulted in a different area from that

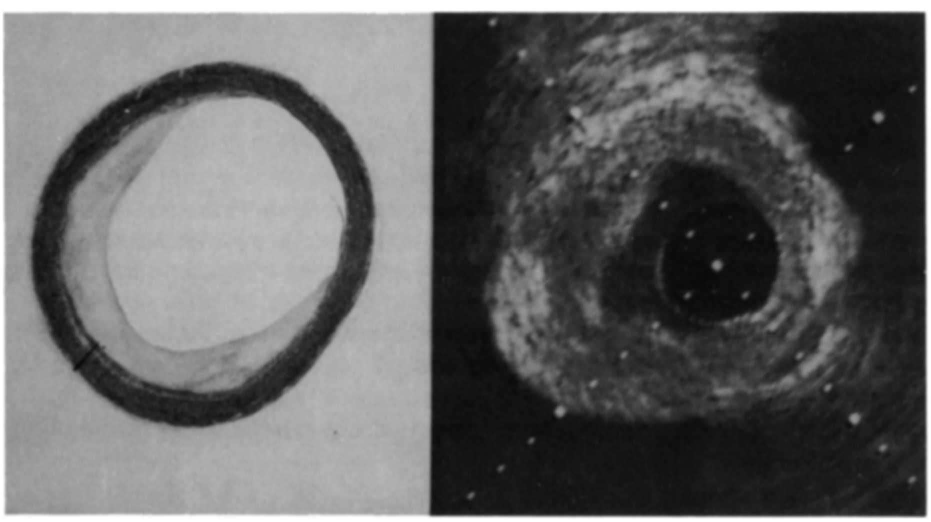

Figure 11. Carotid artery with large fibrotic plaque. Interface between plaque and underlying media is not distinct. **Left,** Histologic specimen; **right,** Ultrasound image.

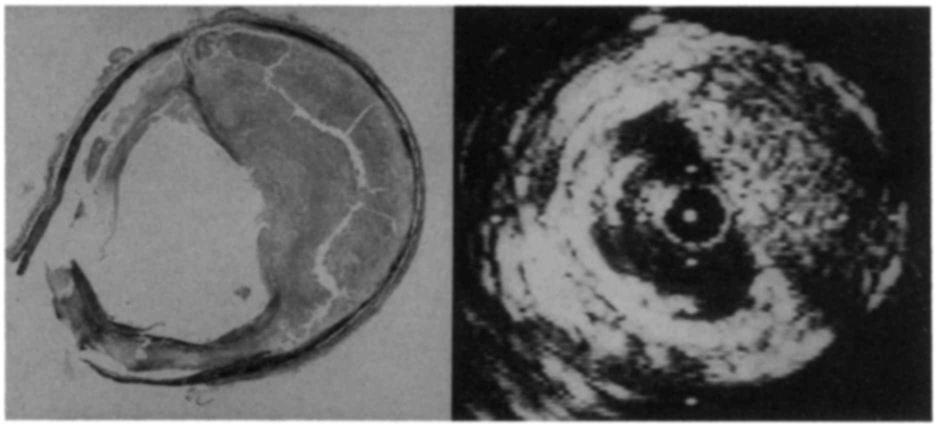

Figure 12. Ruptured necrotic plaque with large thrombus formation. **Left,** Histologic specimen; **right,** Ultrasound image.

measured after pressure-perfusion fixation. However, it was necessary to obtain the arteries and study them freshly because it is not known whether fixation alters the echo characteristics of the various vascular structures. An additional factor could be that the catheter was not completely coaxial to the vessel lumen, a condition that is known to result in an overestimation of the area.

Interface between media and adventitia. The ability to detect an interface between media and adventitia was dependent on the composition of the media as well as that of the adventitia. In elastic arteries, the interface was distinct if there was loose collagen in the adventitia. However, if dense collagen and elastin characterized the adventitia, less of an interface was observed by ultrasound imaging because the tissue characteristics of the media and adventitia were more similar.

In transitional and muscular arteries, the appearance of a distinct interface between media and adventitia was dependent on the location and extent of smooth muscle in the adventitia. Because the media consisted of smooth muscle, no interface was present if appreciable smooth muscle was also scattered throughout the adventitia. However, if adventitial smooth muscle was minimal or was located only at the outer boundary, a distinct interface was still present.

Although one purpose of the present study was to identify distinct patterns of ultrasound images, we found instead, a gradation of patterns with respect to differentiation of media from adventitia.

Intimal morphology. The ability of intravascular ultrasound imaging to delineate intimal details varied with the thickness and morphology of the intima. Relatively normal arteries had only a thin intimal layer on microscopic examination, and, as expected, this could not be identified on the intravascular ultrasound image. Intimal plaque that was highly calcified had a large amount of echo reflectance, producing shadowing. Therefore, even though the luminal area could still be accurately determined, as could the presence of the calcification, shadowing obscured evaluation of the deeper arterial wall components (11).

In several instances there was a clear interface between intimal plaque and the underlying media. This occurred when there was a significant amount of necrotic tissue in the plaque. However, it also occurred in several instances in which the plaque was fibrotic but there was also a dense internal elastic lamina. The presence of thrombus was readily identified, as has been reported previously (19). In most peripheral arteries, there was no clear delineation between the intimal plaque and the underlying media. This finding has important clinical implications if intravascular ultrasound imaging is to be used to determine the thickness and location of intimal plaque before catheter-based interventions.

Limitations. Several limitations were evident when the intravascular ultrasound catheter was evaluated. If the catheter was not directly coaxial to the long axis of the vessel, the calculation of the luminal area was in error. In addition, the ultrasound appearances of the various layers in the vessel wall became indistinct when the catheter was not placed coaxial to the long axis of the vessel or was placed eccentrically within the vessel. The study was performed on peripheral vessels because of the relatively large size of the prototype catheter; however, further investigative work needs to be done with coronary arteries. The transducer had a frequency of 20 MHz; in the future, higher resolution of the different arterial structures may be achieved with more sophisticated, higher frequency transducers.

Conclusions. Intravascular ultrasound imaging is a new method in which high resolution "endovascular" images are obtained from a catheter placed within an artery. Accurate determination of luminal dimensions of vessels is readily obtainable, as is some characterization of arterial wall components. Arterial wall layers are best differentiated in vessels in which components produce acoustic interfaces. Atherosclerotic plaque can be delineated, but its thickness and demarcation from the media cannot always be determined. Calcification of plaque obscures deeper details because of acoustic shadowing.

Intravascular ultrasound imaging may prove useful for

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characterizing and quantitating arterial lesions and for assessing interventional procedures such as angioplasty and atherectomy (9,20,21).

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